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# Chronic hypertension in pregnancy: the impact of ethnicity and superimposed preeclampsia on placental, endothelial and renal biomarkers

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**Author contributions:** LMW and LCC designed the study, with input from all other authors. LMW and CG processed and analysed the samples, in addition to writing the manuscript. PS conducted the statistical analysis with assistance from LMW, KB and LCC. CW, JEM and LCC were site investigators for the study. KB, CNP, JEM and LCC supervised and corrected the manuscript.

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**ABSTRACT**

Black ethnicity is associated with worse pregnancy outcomes in women with chronic hypertension. Pre-existing endothelial and renal dysfunction, and poor placentation may contribute but pathophysiological mechanisms underpinning increased risk are poorly understood. This cohort study aimed to investigate the relationship between ethnicity, superimposed pre-eclampsia and longitudinal changes in markers of endothelial, renal and placental dysfunction in women with chronic hypertension. Plasma concentrations of placental growth factor (PIGF), syndecan-1, renin, aldosterone, and urinary angiotensinogen:creatinine ratio (AGTCR), protein:creatinine ratio (PCR) and albumin:creatinine ratio (ACR) were quantified during pregnancy and postpartum in women with chronic hypertension. Comparisons of longitudinal biomarker concentrations were made using log-transformation and random effects logistic regression allowing for gestation. Of 117 women, superimposed preeclampsia was diagnosed in 21% (n=25), with 24% (n=6) having an additional diagnosis of diabetes. The cohort included 63 (54%) women who self-identified as of Black ethnicity. PIGF concentrations were 67% lower (95% CI -79% to -48%), and AGTCR, PCR and ACR were higher over gestation, in women with subsequent superimposed preeclampsia (compared to those without superimposed preeclampsia). PIGF <100 pg/mL at 20-23.9 weeks' gestation predicted subsequent birthweight <3<sup>rd</sup> centile with 88% sensitivity (95% CI 47%-100%) and 83% specificity (95% CI 70%-92%). Black women had 43% lower renin (95% CI -58% to -23%) and 41% lower aldosterone (95%CI -45% to -15%) concentrations over gestation. Changes in placental (PIGF) and renal (AGTCR/PCR/ACR) biomarkers predated adverse pregnancy outcome. Ethnic variation in the renin-angiotensin-aldosterone system exists in women with chronic hypertension in pregnancy and may be important in treatment selection.

**Key words:** chronic hypertension, pregnancy, renin-angiotensin-aldosterone system, placental growth factor

## INTRODUCTION

Chronic hypertension is estimated to affect 3% of pregnancies(41, 42) and is associated with adverse maternal and perinatal outcome.(5, 6) A recent systematic review by Bramham and colleagues (2014) reported a relative risk of superimposed preeclampsia of 7.7 (95% confidence interval 5.7 to 10.1) compared to the risk of preeclampsia in the general pregnant population.(6) Adverse perinatal outcomes such as stillbirth, fetal growth restriction and prematurity are also more common in women with chronic hypertension and occur independently from a diagnosis of superimposed preeclampsia.(1, 13, 43) Women of Black ethnicity, compared to women of White ethnicity with chronic hypertension, have an increased risk of superimposed preeclampsia,(13) which may be contributory, but greater understanding of the interplay of known biomarkers and ethnicity in women with chronic hypertension may provide insight into pathophysiological mechanisms.

Preeclampsia is characterized by placental and maternal vascular dysfunction.(44) Placental growth factor (PlGF) and syndecan-1 are placental and endothelial biomarkers that have previously been shown to decrease in concentration in the maternal circulation prior to the onset of the clinical signs of preeclampsia.(23, 30, 31) A recent study by Chappell and colleagues (2013) has demonstrated that low PlGF, in women presenting before 35 weeks' gestation with suspected preeclampsia, has high sensitivity and negative predictive value for preeclampsia within 14 days.(12) Diagnosing superimposed preeclampsia in women with chronic hypertension is challenging because gestational progression of hypertension may occur in response to the physiological changes in pregnancy, without the sequelae of preeclampsia. Variation in these biomarkers over gestation in women with chronic hypertension requires further exploration.

The kidney is of critical importance in blood pressure regulation primarily via salt and water homeostasis. A key mechanism underpinning this homeostatic function is the role of the kidney in the systemic renin-angiotensin-aldosterone system (RAAS). Increased glomerular filtration and upregulation of the circulating RAAS are amongst the physiological changes that characterise normotensive pregnancy.(17, 19) Changes in

the RAAS and other renal biomarkers (protein: creatinine ratio (PCR) and albumin: creatinine ratio (ACR)) have been demonstrated in preeclampsia;(7, 15) however there are limited data from longitudinal cohorts examining these biomarkers in pregnant women with chronic hypertension.(4) The impact of ethnicity on these biomarkers in pregnancy also requires exploration. Black women are more likely to have low renin hypertension(40) and ethnic differences in RAAS function in pregnancy may partly explain disparity observed in clinical outcome.(8, 38)

This study aimed to investigate the longitudinal variation in endothelial and renal biomarkers in women with chronic hypertension in pregnancy, and the impact of subsequent superimposed preeclampsia and ethnicity on these biomarkers.

## GLOSSARY

AGTCR	Angiotensinogen: creatinine ratio
ACR	Albumin: creatinine ratio
CHT	Chronic hypertension
ELISA	Enzyme-linked immunosorbent assay
GROW	Gestation adjusted optimal weight
NHS	National Health Service
PCR	Protein: creatinine ratio
PIGF	Placental growth factor
RAAS	Renin-angiotensin-aldosterone system (systemic)
RAS	Renin-angiotensin system (organ-specific)
SPE	Superimposed preeclampsia

## **MATERIALS AND METHODS**

This was a nested cohort study of women with chronic hypertension who participated in the 'Pregnancy And chronic hypertension: NifeDipine vs lAbetalol as antihypertensive treatment' trial between 2014 and 2016. The study was registered with ISRCTN (DOI 10.1186/ISRCTN40973936, [www.isrctn.com](http://www.isrctn.com)) and approved by the UK Research Ethics Committee (REC number 13/EE/0390). The primary results of the randomised controlled trial and the mechanistic comparison of antihypertensive treatment has been presented elsewhere.(48) This cohort study was reported in line with STROBE guidance for observational studies.(20)

### **Study Design**

Women were enrolled at three consultant-led National Health Service (NHS) obstetric units in the United Kingdom (Guy's and St Thomas' NHS Foundation Trust (London), Central Manchester University NHS Foundation Trust (Manchester), and University of Leicester Hospitals NHS Trust (Leicester)). The eligibility criteria included: women with a prenatal diagnosis of chronic hypertension or blood pressure readings  $\geq 140/90$  mm Hg at least four hours apart prior to 20 weeks' gestation requiring antihypertensive treatment, (as defined by the International Society for the Study of Hypertension in Pregnancy classification of hypertensive disorders of pregnancy(47)) between 12 and 27.9 weeks' gestation, singleton pregnancies, aged over 18 years, and the ability to provide informed consent. No formal power calculation was performed, but all women participating in the main trial at the sites afore mentioned, were asked to participate in the sub-study. Participants were asked to provide longitudinal samples of blood and urine at study entry and at follow-up antenatal visits across gestation and at six weeks postpartum. Baseline demographic and antenatal booking data were collected at enrollment. Ethnicity (Black versus non-Black) was determined by self-report of a parent or grandparent who was African/Caribbean (Black ethnicity) or not (non-Black ethnicity, which included women of European and Asian family origin). Blood pressure readings taken at all subsequent antenatal visits and daily during hospital admissions were recorded in addition to other maternal and perinatal outcome data (superimposed preeclampsia, mode of delivery, gestation at delivery, pregnancy loss, birthweight, birthweight centile and neonatal unit admission). The

diagnosis of superimposed preeclampsia utilised the definitions from the American Congress of Obstetrics and Gynaecology;(37) the diagnosis was confirmed by two clinicians and was either defined as chronic hypertension with new-onset proteinuria in those without chronic kidney disease or a sudden increase in proteinuria in those with chronic kidney disease, and an increase in hypertension. Women with PCR >30 mg/mmol at study entry (likely indicative of chronic kidney disease) were excluded from the analysis of urinary biomarkers. Customised birthweight centiles were calculated using the GROW formula with adjustment for maternal height, maternal weight, maternal ethnicity, parity, infant sex, infant birthweight and gestation at birth (version 6.7.5.1 (2014)).(24) An international consensus statement has now determined that birthweight below the 3<sup>rd</sup> centile represents fetal growth restriction and birthweight below the 10<sup>th</sup> centile represents small for gestational age.(25)

### **Analysis of Samples**

Venous blood and midstream urine samples were placed on ice immediately after collection, spun at 1400xg for 10 minutes and stored at -80°C within four hours. The following tests were conducted without knowledge of clinical outcomes.

*Placental and endothelial biomarkers:* Plasma PIGF and plasma syndecan-1 concentrations were measured on all antenatal samples. PIGF was quantified using the Triage PIGF Test (Alere, San Diego, USA) according to the manufacturer's instructions.(12) Syndecan-1 concentrations were measured using a solid phase sandwich ELISA kit for quantification of human syndecan-1 (Abnova, Taipei City, Taiwan), in accordance with manufacturer's instructions.(45)

*Renal biomarkers:* Plasma renin and aldosterone concentration, and urinary AGTCR, PCR, and ACR were quantified for all samples (antenatal and postpartum). Plasma renin and aldosterone were measured using Diasorin Liaison direct renin and aldosterone reagents respectively (Diasorin, Wokingham, UK), run on the Liaison chemiluminescence analyser.(9)

Urinary angiotensinogen concentrations were determined using a solid phase sandwich ELISA kit (Immuno-Biological Laboratories, Gunma, Japan) according to manufacturer's instructions.(27) The urinary angiotensinogen concentrations were then normalised to urinary creatinine to provide AGTCR. Urinary creatinine, protein and albumin reagents were supplied by Siemens Healthcare Diagnostics Ltd (Camberley, UK). Urinary creatinine reacted with picric acid under alkaline conditions to form a red Janovsky complex (the Jaffé reaction).(34) The initial rate of absorbance change was measured at a wavelength of 505 nm and compared to that of a known calibrant. This was directly proportional to the concentration of creatinine in the sample. A blank reaction rate was performed using reagent 1 (sodium hydroxide, before picrate addition) to minimise interference from bilirubin. Urinary protein forms a blue coloured complex with pyrogallol red, under acid conditions and in the presence of molybdate ions.(22) The absorbance of this complex was measured at 596 nm, and related to that of a previous calibration assay. Urine albumin was measured using a polyethylglycol enhanced immunoturbidimetric method a commonly used assay technique.(10) The sample was diluted and then reacted with antiserum to form a precipitate that was measured turbidimetrically at 340nm. Urine albumin was measured on the Siemens Advia 2400 analyser.

## Statistical Analysis

The statistical software Stata version 14 (StataCorp, College Station, Texas) and GraphPad Prism 7 (Graph Pad Software, San Diego, California) were used for all analyses. The investigation was divided into two parts. Analysis A compared baseline characteristics, clinical outcomes and endothelial and renal biomarkers between women with chronic hypertension who subsequently developed superimposed preeclampsia and women with chronic hypertension who did not develop superimposed preeclampsia during their pregnancy. Analysis B compared baseline characteristics, clinical outcomes and endothelial and renal biomarkers between women with chronic hypertension self-identifying as of Black ethnicity with women with chronic hypertension self-identifying as of non-Black ethnicity. An additional analysis examined the relationship between each biomarker and birthweight centile. Baseline characteristics and clinical outcomes were



compared between subgroups in analyses A and B using t-tests or Mann-Whitney test for continuous variables depending on the distributions and Fisher's exact test for categorical variables. Mean post-enrollment systolic and diastolic blood pressure were calculated using the trapezium method of analysing the area under the curve for each woman. Variation in longitudinal endothelial and renal biomarkers for analyses A and B was assessed using random effects GLS regression with robust standard errors(3) on log-transformed data allowing for gestation effects. Logged estimates with 95% confidence intervals of the geometric means and of their ratios to the reference group in each analysis were calculated. Standard mathematic methods converted these to percentage differences between groups: percent difference =  $(\exp[\text{parameter}] - 1) \times 100\%$ . An interaction test examining the correlation between ethnicity, diagnosis of superimposed preeclampsia and gestation was performed using a random-effects regression model, wherever significant ethnic differences in biomarker concentration were demonstrated.

## RESULTS

The cohort recruited to the study included 121 women with singleton pregnancies and chronic hypertension (Figure 1). Longitudinal samples (332 in total) were obtained in 117 (97%) women; outcome data were unavailable for four (3.3%) women (two women lost to follow-up and two withdrew from the study). For analysis A, the cohort was divided into women diagnosed with superimposed preeclampsia and compared with women who were not diagnosed with superimposed preeclampsia (n=25 versus n=92) and for analysis B, the cohort was divided into women self-identifying as of Black ethnicity versus women self-identifying as of non-Black ethnicity (n=63 versus n=54).

The baseline demographics of the women who provided samples for the analyses are detailed in Table 1. The women who developed superimposed preeclampsia, compared to those who did not, were younger (33 years versus 36 years;  $p$  0.04), and a higher proportion of the women who developed superimposed preeclampsia, compared to those who did not, had pre-existing diabetes mellitus (24% versus 6.5%;  $p$  0.02). Fewer black women were nulliparous, compared to non-Black women (6.3% versus 33%;  $p$  0.0003), and the

diastolic blood pressure was higher in Black women than non-Black women at antenatal booking (88 mmHg versus 85 mmHg;  $p$  0.03). Otherwise the groups were comparable at baseline.

Maternal and perinatal outcomes for the cohort as a whole, in addition to the subgroups within analysis A (superimposed preeclampsia versus chronic hypertensive controls) and B (Black women versus non-Black women), are shown in Table 2 and 3. There were no differences in outcome between labetalol and nifedipine arms of the trial. Superimposed preeclampsia was diagnosed in 21% ( $n=25$ ) of the whole cohort of women with chronic hypertension, with 31% ( $n=36$ ) of infants being born before 37 weeks' gestation and 91% ( $n=106$ ) livebirths. Of the 106 livebirths, 29% ( $n=31$ ) had a birthweight below the 10<sup>th</sup> centile ( $n=18$  of these infants were born before 37 weeks' gestation), 15% ( $n=16$ ) had a birthweight below the 3<sup>rd</sup> centile, and 23% ( $n=25$ ) required admission to the neonatal unit.

Within the subgroups, the greatest differences in maternal and perinatal outcome were seen in the women with chronic hypertension who developed superimposed preeclampsia compared to chronic hypertensive controls. The mean systolic (137 mmHg versus 132 mmHg;  $p$  0.005) and mean diastolic (87 mmHg versus 84 mmHg;  $p$  0.002) blood pressures were higher in the women with superimposed preeclampsia compared to those with no superimposed preeclampsia. Women diagnosed with superimposed preeclampsia were more likely to have an emergency Caesarean birth, compared to those without superimposed preeclampsia (64% versus 42%;  $p$  0.03). Additionally, preterm birth before 37 weeks' gestation occurred more frequently in women with superimposed preeclampsia than without (56% versus 24%;  $p$  0.003). The infants born to mothers who were diagnosed with superimposed preeclampsia, compared to those who were not, had lower birthweights (2270g versus 3020g;  $p$  <0.0001) and were more likely to be admitted to the neonatal unit (52% versus 14%;  $p$  0.0007). The only significant difference in maternal and perinatal outcome found between the women of Black ethnicity and those of non-Black ethnicity, was higher mean post-enrollment diastolic blood pressure (Black women: 86 mmHg versus non-Black women: 83 mmHg;  $p$  0.03).

219

220 **Endothelial biomarkers**

221 The longitudinal changes in PlGF and syndecan-1 are displayed in Figures 2, 3 and 4. Analysis A  
222 (superimposed preeclampsia versus no superimposed preeclampsia) demonstrated that PlGF concentration  
223 increased across gestation in the women who did not develop SPE to a peak mean value of 363 pg/mL at 28  
224 to 31.9 weeks' gestation, but in the women who developed subsequent SPE the peak mean PlGF was 105  
225 pg/mL and occurred at 20 to 23.9 weeks' gestation (Figure 2). Comparative analysis demonstrated that the  
226 PlGF concentrations were 67% lower (95% confidence interval -79% to -48%;  $p < 0.001$ ) in women who  
227 developed superimposed preeclampsia compared to those who did not. In analysis B, PlGF concentration  
228 increased across gestation in women of Black ethnicity and women of non-Black ethnicity (Figure 2). There  
229 was no overall difference in concentrations between groups (-2%; 95% confidence interval -43% to 68%;  
230  $p = 0.94$ ).

231

232 Given the high proportion of infants born below the 3<sup>rd</sup> and 10<sup>th</sup> centile in this cohort of women with chronic  
233 hypertension, an additional analysis assessed the gestational changes in each biomarker and birthweight  
234 centile. The only biomarker demonstrating a significant association with birthweight centile category ( $< 3^{\text{rd}}$ ,  
235 3<sup>rd</sup> to 10<sup>th</sup> and  $> 10^{\text{th}}$ ) was PlGF (Figure 3). PlGF  $< 100$  pg/mL at 20 to 23.9 weeks' gestation to predict  
236 subsequent birthweight  $< 3^{\text{rd}}$  centile demonstrated 88% sensitivity (95% confidence interval 47% to 100%),  
237 83% specificity (95% confidence interval 70% to 92%), and the negative predictive value was 98% (95%  
238 confidence interval 88% to 100%).

239

240 Syndecan-1 concentrations increased significantly across gestation in all subgroups ( $p < 0.0001$ ) (Figure 4).  
241 Postpartum syndecan-1 concentrations were 83% lower (95% confidence interval -87% to -78%;  $p < 0.001$ )  
242 than antenatal syndecan-1 concentrations. No significant differences were found in syndecan-1  
243 concentrations either in women with chronic hypertension who did and did not develop superimposed

preeclampsia (-5%; 95% confidence interval -24% to 18%;  $p=0.62$ ), or in women with chronic hypertension of Black or non-Black ethnicity (-5%; 95% confidence interval -22% to 15%;  $p=0.59$ ).

#### Renal biomarkers

Plasma renin concentrations did not vary significantly over the gestation studied (12 to 35.9 weeks) (Figure 5); however antepartum renin concentrations were significantly higher than in samples taken six weeks postpartum ( $p < 0.0001$ ). In the women who developed superimposed preeclampsia, the mean renin concentrations at each gestational time point tended to be higher than in women who did not develop superimposed preeclampsia until 32 to 35.9 weeks' gestation. However, these differences were not statistically significant when longitudinal measures were compared (34%; 95% confidence interval -2% to 82%;  $p=0.06$ ). In Black women with chronic hypertension with or without superimposed preeclampsia, the mean renin concentrations were significantly lower across gestation compared to women of non-Black ethnicity (-43%; 95% confidence interval -58% to -23%;  $p < 0.001$ ). Further analysis explored the differences between gestational renin concentrations and six-week postpartum renin concentrations. In women of Black ethnicity, postpartum renin concentrations were 81% lower (95% confidence interval -91% to -58%;  $p < 0.001$ ) compared with antenatal concentrations and in non-Black women the postpartum renin concentrations were 54% lower (95% confidence interval -73% to -22%;  $p=0.004$ ) than antenatal values in this group. An interaction test examined the potential relationship between ethnicity, gestation and diagnosis of superimposed preeclampsia and did not demonstrate a significant correlation.

Aldosterone concentrations were 57% higher (95% confidence interval 43% to 68%;  $p < 0.001$ ) in pregnancy than at six weeks postpartum. No significant difference was found in aldosterone concentrations between the women who did and did not develop superimposed preeclampsia (5%; 95% confidence interval -20% to 39%;  $p=0.73$ ) (Figure 6). In the women of Black ethnicity compared to those of non-Black ethnicity who did or did not develop superimposed preeclampsia, aldosterone concentrations in pregnancy were 31% lower (95% confidence interval -45% to -15%;  $p=0.001$ ). There was no difference in aldosterone concentrations

between ethnic groups at the six-week postpartum timepoint (13%; 95% confidence interval -46% to 131%;  $p=0.72$ ). Across gestation, aldosterone concentrations increased by 70% in the women of non-Black ethnicity (95% confidence interval 39% to 209%;  $p<0.001$ ); however in the Black women no significant increase in aldosterone concentrations was demonstrated across gestation (13%; 95% confidence interval -7% to 37%;  $p=0.23$ ). An interaction test explored the potential association of ethnicity, gestation and diagnosis of superimposed preeclampsia and did not demonstrate a significant correlation. Aldosterone: renin ratio was calculated and compared for analysis A and B, but no association was seen.

Women with chronic kidney disease were excluded from the analysis of the urinary biomarkers ( $n=11$ ). Urinary AGTCR increased across gestation and was significantly lower at six weeks postpartum (-79%; 95% confidence interval -86% to -69%;  $p<0.001$ ). In the women with superimposed preeclampsia, compared to chronic hypertensive controls, the AGTCR was 63% higher across gestation (95% confidence interval 10% to 142%;  $p=0.02$ ) (Figure 7). There was no difference between the AGTCR in women of Black or non-Black ethnicity (12%; 95% confidence interval -22% to 61%;  $p=0.53$ ). No correlation between the systemic RAAS (plasma renin and aldosterone) or the intra-renal RAS (AGTCR) was found.

PCR increased across gestation in all subgroups (51%; 95% confidence interval 24% to 84%;  $p<0.001$ ) and returned to levels comparable to the 12 to 16.9 week mean ratio by six weeks postpartum. PCR was overall significantly higher across gestation in the women who subsequently developed superimposed preeclampsia compared to those who did not (29%; 95% confidence interval 5% to 57%;  $p=0.01$ ) (Figure 8). There was no significant difference in PCR across gestation in Black and non-Black women (4%; 95% confidence interval -14% to 24%;  $p=0.71$ ).

ACR did not vary significantly across gestation in the cohort as a whole (20%; 95% confidence interval -10% to 59%;  $p=0.22$ ). However, in the women who were subsequently diagnosed with superimposed preeclampsia compared to chronic hypertensive controls, ACR was higher (123%; 95% confidence interval

67% to 197%;  $p < 0.001$ ) (Figure 9). There was no difference in ACR when women of Black and non-Black ethnicity were compared (10%; 95% confidence interval -19% to 50%;  $p = 0.55$ ).

## DISCUSSION

This study has confirmed that low PIGF across gestation in women with chronic hypertension in pregnancy predates the clinical presentation of superimposed preeclampsia and fetal growth restriction. To our knowledge, this is the first study in women with chronic hypertension to demonstrate the potential utility of PIGF as a predictive tool for fetal growth restriction; with further validation, PIGF concentrations  $< 100$  pg/mL at 20 to 23.9 weeks' gestation may aid risk stratification and timing of ultrasound surveillance in this group. Furthermore, the potential utility of PIGF in the diagnosis and prediction of superimposed preeclampsia in women with chronic hypertension, a group in whom the diagnosis is most challenging given the pre-existing hypertension, is demonstrated. Low PIGF concentrations across gestation in women who develop preeclampsia has been demonstrated in women without chronic hypertension.(31) It has been proposed that the imbalance in angiogenic markers observed in women with preeclampsia was greater than observed in women with superimposed preeclampsia,(14) but the findings of our study support comparable pathophysiology underpinning preeclampsia in women with and without chronic hypertension and reported elsewhere.(35)

The high incidence of adverse maternal and perinatal outcomes in women with chronic hypertension in pregnancy is highlighted by this study with 21% of women developing superimposed preeclampsia, comparable with the findings of other studies ranging from 17 to 25%.(33, 39, 41, 42) The incidence of superimposed preeclampsia in this cohort may relate in part to the number of participants with diabetes, as demonstrated by the significant difference in the proportion of women with diabetes and chronic hypertension that developed superimposed preeclampsia compared to those who did not (24% versus 6.5%;  $p = 0.02$ ). Women with chronic hypertension who did not develop superimposed preeclampsia were also at increased risk of adverse perinatal outcome compared to background incidence, but a significantly greater

proportion of adverse perinatal outcomes were seen in the women who developed superimposed preeclampsia.

Syndecan-1 is a transmembrane heparin sulphate proteoglycan expressed on the extracellular, luminal surface of epithelial cells and syncytiotrophoblasts, forming part of the glycocalyx of these cells. Syndecan-1 is involved in the regulation of cell adhesion, proliferation, motility, intra-cellular signalling and angiogenesis.(46) Our findings confirm circulating syndecan-1 concentrations increase across gestation as previously demonstrated;(23) however in this study syndecan-1 concentrations did not differ significantly in women with chronic hypertension who developed subsequent superimposed preeclampsia compared with chronic hypertensive controls. This finding is in contrast to results reported by Gandley and colleagues (2016) who reported that plasma syndecan-1 concentrations at 20 weeks' gestation were significantly lower in women who developed subsequent preeclampsia (174 ng/mL versus 272 ng/mL;  $p < 0.05$ ). (23) These differences may relate to differing pathophysiology underpinning preeclampsia in women with chronic hypertension compared to women without chronic hypertension; although the relative contributions of angiogenic imbalance and other factors is uncertain, these results support low PlGF concentrations having a better association with subsequent disease rather than syndecan-1.(14) Further exploration of the role of syndecan-1 in the pathophysiology of placental disease in different populations is required to determine the clinical utility of this biomarker.

The role of the systemic RAAS in the pathophysiology of placental disease in women with chronic hypertension in pregnancy is unclear. No significant variations in plasma renin or aldosterone concentrations were found across gestation in the women who did and did not develop superimposed preeclampsia; this may be driven by the expected Angiotensin II inhibitory feedback on renin release, which is independent of angiotensinogen substrate production. Women with preeclampsia demonstrate greater sensitivity to Angiotensin II, compared with normotensive pregnant women; it is possible the cellular RAS pathway through which the increased sensitivity is demonstrated, is independent of systemic substrates.(29) Previous

studies have demonstrated that the RAAS is upregulated in normotensive pregnancy and our study confirms that this upregulation occurs in both the Black and non-Black women with chronic hypertension, though it is unclear if this is in response to, or the cause of other physiological changes that occur in pregnancy such as increased plasma volume and vasodilation.(2) Brown and colleagues (1997) performed a cross-sectional study and found that women diagnosed with preeclampsia had lower plasma concentrations of renin and aldosterone compared to normotensive pregnant women.(7) Another longitudinal study by August and colleagues (1990) in 25 pregnant women with chronic hypertension, demonstrated reduced plasma renin activity and lower urinary aldosterone concentrations in those who developed subsequent superimposed preeclampsia compared to those who did not.(4) Importantly our study confirms that systemic renin and aldosterone concentrations in Black women with chronic hypertension are lower across gestation compared to non-Black women in keeping with the non-pregnant state, and any future investigation of the role of the systemic RAAS in pregnancy should account for this ethnic variation.

It is interesting that although there is variation in the systemic RAAS between ethnic groups, no variation in the intrarenal RAS was observed, nor was any relationship between systemic and intrarenal identified. Increased urinary AGTCR were found across gestation in women who developed superimposed preeclampsia compared to those who did not. Pathological upregulation of the intrarenal RAS in preeclampsia would be consistent with findings outside pregnancy; an activated intrarenal RAS has been reported in the progression of renal injury in diabetic and membranous nephropathy.(28) However, our findings do not agree with a cross-sectional study reported by Yilmaz and colleagues (2009), who compared time-of-disease AGTCR in 30 women with preeclampsia with 30 normotensive pregnant women. They found that urinary AGTCR were lower in women with preeclampsia,(49) which may represent variation specific to their sample. This study did, however, report a positive correlation between urinary AGTCR, high blood pressure and proteinuria, and concluded that 'local RAS activation in the kidneys may be one of the contributing factors in the development of preeclampsia'.(49) In this respect our findings concur, but further investigation is required to establish the importance of the intrarenal RAS in the pathophysiology of superimposed preeclampsia.



374

375 PCR and ACR were higher across gestation in women with chronic hypertension who developed subsequent  
 376 superimposed preeclampsia compared to those who did not. This may represent underlying nephropathy in  
 377 this group of women with chronic hypertension, as described by Crews and colleagues (2010).(16) However,  
 378 another study by Poon and colleagues (2008) found higher first trimester ACR was associated with  
 379 subsequent development of preeclampsia,(36) so it may be that the association of higher PCR/ACR from the  
 380 first trimester is indicative of endothelial dysfunction with subsequent development of superimposed  
 381 preeclampsia. The clinical utility of these findings are unclear, as proteinuria is known to increase in  
 382 normotensive pregnancy and identifying when protein excretion is abnormal is problematic.(18, 32) It is also  
 383 notable that the mean PCR and ACR values across gestation in the women in our cohort who developed  
 384 subsequent superimposed preeclampsia were below 30 mg/mmol and 8 mg/mmol respectively (prior to  
 385 diagnosis), which are currently utilised for clinical diagnosis.(47)

386

387 In this study, the proportion of the Black women developing superimposed preeclampsia was lower than the  
 388 proportion of non-Black women (14% versus 30%;  $p=0.07$ ), though this was not significant and may relate in  
 389 part to the higher proportion of multiparous women in the Black group. The significant difference in booking  
 390 and mean post-enrollment diastolic blood pressures in women of Black ethnicity, compared to non-Black,  
 391 suggests potential ethnic variation in disease severity, which has been demonstrated in the non-pregnant  
 392 population.(11, 21, 26) Variation in the systemic RAAS may play a role in ethnic disparity in disease severity;  
 393 the incidence of SPE in Black women is lower within this cohort compared to the increased incidence of SPE  
 394 in Black women observed in other cohorts and this may have had an impact on the findings of these  
 395 analyses. Our study has highlighted differences in the RAAS, in particular a third trimester increase in  
 396 aldosterone concentrations that is present in the non-Black women, but not in the Black women and these  
 397 findings warrant further exploration.

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The strengths of this study include multicentre recruitment of a wide ethnic mixture of women with chronic hypertension in pregnancy. Longitudinal sampling has allowed assessment of variation of biomarkers across gestation prior to development of adverse outcome. In contrast, a limitation of the study was the absence of time-of-disease sampling, preventing comparison of each biomarker at the time of clinical diagnosis of superimposed preeclampsia, and the findings of this study would require validation in a much larger cohort. The high proportion of women with diabetes in this cohort may also have impacted the results of analysis A and it is not possible to fully elucidate the potential impact of hyperglycaemia on our findings. Additionally, although some participants may have had white coat hypertension, 24-hour ambulatory monitoring is not routinely performed during pregnancy and would usually only have been confirmed after pregnancy. Future research should aim to examine longitudinal and time of disease biomarker concentrations, with simultaneous quantification of other biomarkers that are involved in pathophysiological pathways such as the RAAS. This would provide further explanation of the mechanisms underpinning increased adverse maternal and perinatal outcome in pregnant women with chronic hypertension.

## **PERSPECTIVES AND SIGNIFICANCE**

The incidence of chronic hypertension in pregnancy is increasing with rising maternal age and obesity. The prevalence of chronic hypertension in women aged below 50 years is greater in those of Black ethnicity, compared to other ethnicities. Adverse maternal and perinatal outcomes are associated with chronic hypertension and the mechanisms behind these increased risks are poorly understood. In this study, changes in placental and renal biomarkers predate development of superimposed preeclampsia. Low plasma PIGF has a strong association with subsequent fetal growth restriction in women with chronic hypertension and ethnic variation in RAAS biomarkers may relate to disparity in outcome. Further research into the physiology underpinning these differences is warranted.

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## **ETHICAL APPROVAL**

The protocol and other study literature was approved by the UK Research Ethics Committee (REC number 13/EE/0390).

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## **DISCLOSURES**

Professor Nelson-Piercy reports personal fees from Alliance Pharmaceuticals, personal fees from UCB Pharmaceuticals, LEO Pharmaceuticals, Sanofi Aventis and Warner Chilcott all of which relate to work outside the submitted research. The other authors report no disclosures.

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**FIGURE LEDGENDS:**

**Figure 1** Overview flow of study participants including grouping for analyses A and B

CHT= chronic hypertension, SPE=superimposed preeclampsia

**Figure 2** Placental growth factor concentrations across gestation in pregnant women with chronic hypertension. Lower limit of detection=12pg/mL. Comparison A: superimposed preeclampsia (SPE) versus no SPE. Comparison B: women of Black ethnicity versus non-Black ethnicity Variation in longitudinal PIGF concentration for analyses A and B was assessed using random effects GLS regression with robust standard errors on log-transformed data allowing for gestation effects.

**Figure 3** Placental growth factor concentration and infant birthweight centile. Lower limit of detection=12pg/mL.  
A: Placental growth factor across gestation in pregnant women with chronic hypertension divided by birthweight centile categories (measured using random effects GLS regression with robust standard errors on log-transformed data allowing for gestation effects)  
B: Box plot of placental growth factor concentration at 20 to 23.9 weeks and 24 to 29.9 weeks' gestation divided by infant birthweight centile category

**Figure 4** Syndecan-1 concentrations across gestation in pregnant women with chronic hypertension. Comparison A: superimposed preeclampsia (SPE) versus no SPE. Comparison B: women of Black ethnicity versus non-Black ethnicity. Variation in longitudinal Syndecan-1 concentration for analyses A and B was assessed using random effects GLS regression with robust standard errors on log-transformed data allowing for gestation effects.

**Figure 5** Renin concentrations across gestation in pregnant women with chronic hypertension. Comparison A: superimposed preeclampsia (SPE) versus no SPE. Comparison B: women of Black ethnicity versus non-Black ethnicity. Variation in longitudinal Renin concentration for analyses A and B was assessed using random effects GLS regression with robust standard errors on log-transformed data allowing for gestation effects.

**Figure 6** Aldosterone concentrations across gestation in pregnant women with chronic hypertension. Comparison A: superimposed preeclampsia (SPE) versus no SPE. Comparison B: women of Black ethnicity versus non-Black ethnicity. Variation in longitudinal Aldosterone concentration for analyses A and B was assessed using random effects GLS regression with robust standard errors on log-transformed data allowing for gestation effects.

**Figure 7** Urinary angiotensinogen: creatinine concentrations across gestation in pregnant women with chronic hypertension. Comparison A: superimposed preeclampsia (SPE) versus no SPE. Comparison B: women of Black ethnicity versus non-Black ethnicity. Variation in longitudinal urinary angiotensinogen: creatinine ratio for analyses A and B was assessed using random effects GLS regression with robust standard errors on log-transformed data allowing for gestation effects. Women with chronic kidney disease were excluded from these analyses.

**Figure 8** Urinary protein: creatinine ratio concentrations across gestation in pregnant women with chronic hypertension. Comparison A: superimposed preeclampsia (SPE) versus no SPE (samples taken prior to the diagnosis of superimposed preeclampsia). Comparison B: women of Black ethnicity versus non-Black ethnicity. Variation in longitudinal urinary protein: creatinine ratio for analyses A and B was assessed using random effects GLS regression with robust standard errors on log-transformed data allowing for gestation effects. Women with chronic kidney disease were excluded from these analyses.

623 **Figure 9** *Urinary albumin: creatinine ratio concentrations across gestation in pregnant women with chronic*  
624 *hypertension. Comparison A: superimposed preeclampsia (SPE) versus no SPE (samples taken prior to the*  
625 *diagnosis of superimposed preeclampsia). Comparison B: women of Black ethnicity versus non-Black*  
626 *ethnicity. Variation in longitudinal urinary albumin: creatinine ratio for analyses A and B was assessed using*  
627 *random effects GLS regression with robust standard errors on log-transformed data allowing for gestation*  
628 *effects. Women with chronic kidney disease were excluded from these analyses.*

629 **TABLES:**630 **Table 1 Baseline demographics for the cohort**

Characteristic	All CHT n=117	SPE n=25	CHT without SPE n=92	Black ethnicity n=63	Non-Black ethnicity n=54
<b>Age at study entry, years</b> mean (SD)	35 (5)	33* (6)	36 (5)	36 (5)	35 (6)
<b>Body mass index, Kg/m<sup>2</sup></b> mean (SD)	31 (6.2)	31 (5.8)	31 (6.3)	32 (5.5)	30 (6.9)
<b>Nulliparity</b> number (%)	22 (19%)	7 (28%)	15 (16%)	4* (6.3%)	18 (33%)
<b>Smoker</b> number (%)	1 (0.9%)	0	1 (1.1%)	0	1 (1.9%)
<b>Booking blood pressure, mmHg</b> median (IQR)					
Systolic	135 (126 to 142)	134 (127 to 140)	136 (126 to 142)	139 (126 to 147)	134 (126 to 140)
Diastolic	88 (81 to 92)	85 (82 to 90)	88 (81 to 92)	88* (84 to 96)	85 (80 to 90)
<b>Renal disease</b> number (%)	10 (8.5%)	3 (12%)	7 (7.6%)	4 (6.3%)	6 (11%)
<b>Diabetes mellitus</b> number (%)	12 (10%)	6* (24%)	6 (6.5%)	7 (11%)	5 (9.3%)
<b>Centre</b>					

# Biomarker variation in chronic hypertensive pregnancy

number (%)					
Guy's and St Thomas' NHS Foundation Trust	73 (62%)	10 (40%)	64 (70%)	48 (76%)	25 (46%)
Central Manchester University Hospitals NHS Foundation Trust	29 (25%)	8 (32%)	21 (23%)	13 (21%)	16 (30%)
University Hospitals of Leicester NHS Trust	15 (13%)	7 (28%)	8 (8.7%)	2 (3.2%)	13 (24%)
<b>Assigned antihypertensive treatment</b> number (%)	94 (80%)	19 (76%)	75 (82%)	52 (83%)	42 (78%)
Labetalol	47 (40%)	6 (24%)	41 (45%)	25 (40%)	22 (41%)
Nifedipine	47 (40%)	13 (52%)	34 (37%)	27 (43%)	20 (37%)

631 CHT= chronic hypertension, SPE=superimposed preeclampsia, SD= standard deviation, IQR= interquartile

632 range

633 \*denotes characteristics that are significantly different between the compared subgroups

634

635 **Table 2 Maternal outcomes**

Outcome	All CHT n=117	SPE n=25	CHT without SPE n=92	Black ethnicity n=63	Non-Black ethnicity n=54
<b>Mean blood pressure per woman<sup>†</sup>, mmHg</b> median (IQR)					
Systolic	133 (127 to 137)	137* (131 to 142)	132 (127 to 136)	134 (127 to 140)	132 (128 to 134)
Diastolic	84 (81 to 89)	87* (84 to 93)	84 (81 to 88)	86* (82 to 90)	83 (81 to 86)
<b>Incidence of severe hypertension<sup>‡</sup>, days</b> number (%)					
0	44 (38%)	7 (28%)	37 (40%)	20 (32%)	24 (44%)
1	30 (26%)	4 (16%)	26 (28%)	14 (22%)	16 (30%)
2	15 (12%)	1 (4%)	14 (15%)	10 (16%)	5 (9.3%)
≥3	28 (24%)	13* (52%)	15 (16%)	19 (30%)	9 (17%)
<b>Superimposed preeclampsia</b> number (%)	25 (21%)	25 (100%)	92 (0%)	9 (14%)	16 (30%)
<b>Mode of delivery</b> number (%)					
Spontaneous vaginal delivery	39 (34%)	4 (16%)	35 (39%)	20 (32%)	19 (35%)
Assisted vaginal delivery	6 (5.2%)	3 (12%)	3 (3.3%)	4 (6.5%)	2 (3.7%)

# Biomarker variation in chronic hypertensive pregnancy

Elective Caesarean section	16 (14%)	2 (8%)	14 (16%)	6 (9.7%)	10 (19%)
Emergency Caesarean section	54 (47%)	16* (64%)	38 (42%)	32 (52%)	22 (41%)
<b>Gestation at delivery, weeks</b> median (IQR)	38 (35.9 to 39)	34.7* (30.3 to 37.9)	38.3 (37 to 39.3)	37.9 (35.6 to 39)	38.0 (36 to 39.4)
<b>Pre-term birth &lt;37 weeks</b> number (%)	36 (31%)	14* (56%)	22 (24%)	19 (30%)	17 (31%)
<b>Perinatal outcome</b> number (%)					
Livebirth	106 (91%)	23 (92%)	83 (91%)	56 (89%)	50 (93%)
Miscarriage	4 (3.4%)	0	4 (4.3%)	3 (4.8%)	1 (1.9%)
Termination of pregnancy	3 (2.6%)	1 (4%)	2 (2.2%)	2 (3.2%)	1 (1.9%)
Stillbirth	4 (3.4%)	1 (4%)	3 (3.2%)	2 (3.2%)	2 (3.7%)

CHT= chronic hypertension, SPE=superimposed preeclampsia

\*denotes outcomes that are significantly different between the compared groups

†Mean blood pressure of all antenatal blood pressures during study participation

‡Severe hypertension= systolic blood pressure  $\geq 160$  mmHg and/or diastolic blood pressure  $\geq 110$  mmHg

641 **Table 3 Perinatal outcomes**

Outcome	All CHT n=106	SPE n=23	CHT without SPE n=83	Black ethnicity n=56	Non-Black ethnicity n=50
<b>Birthweight, g</b> Median (IQR)	2920 (2360 to 3250)	2270* (1320 to 2840)	3020 (2690 to 3440)	2980 (2520 to 3180)	2930 (2560 to 3400)
<b>Birthweight &lt;10<sup>th</sup> centile</b> number (%)	31 (29%)	13* (52%)	18 (22%)	17 (30%)	14 (28%)
<b>Birthweight &lt;3<sup>rd</sup> centile</b> number (%)	16 (15%)	10* (40%)	6 (7.2%)	9 (16%)	7 (14%)
<b>Neonatal unit admission</b> number (%)	25 (23%)	12* (52%)	13 (14%)	12 (21%)	13 (26%)

642 *CHT= chronic hypertension, SPE=superimposed preeclampsia*643 *\*denotes outcomes that are significantly different between the compared groups*

644



Total enrolled to study  
n=121

Outcome data unavailable n=4

Total providing longitudinal samples  
n=117

Analysis A:  
Women who developed superimposed  
pre-eclampsia (SPE) versus CHT without SPE

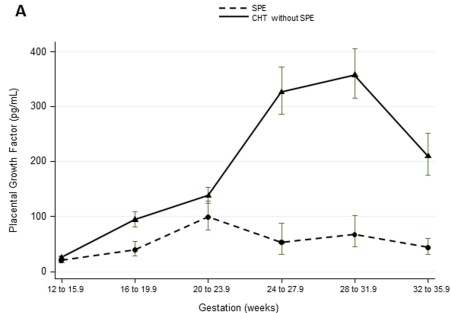
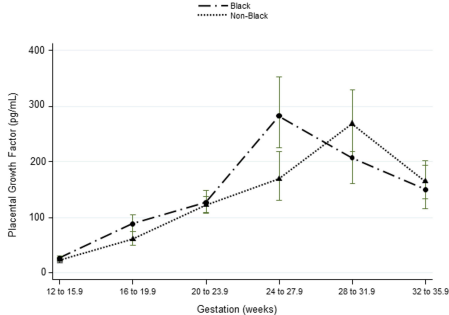
Analysis B:  
Women of Black ethnicity versus  
non-Black ethnicity

SPE  
n=25

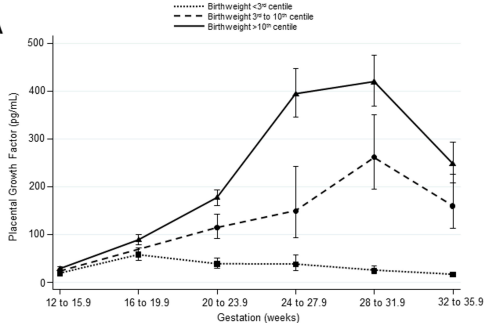
CHT without SPE  
n=92

Black ethnicity  
n=63

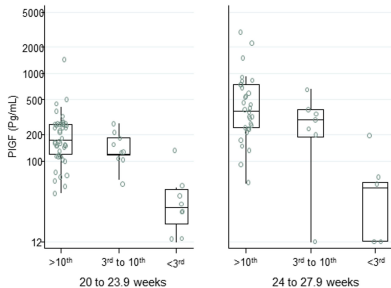
Non-Black ethnicity  
n=54

**A****B**

A



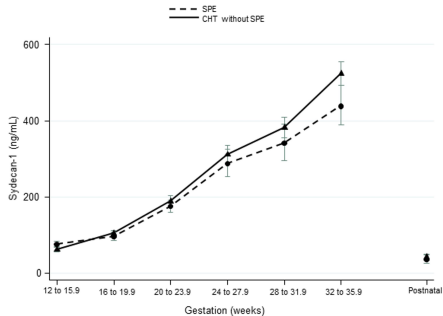
B



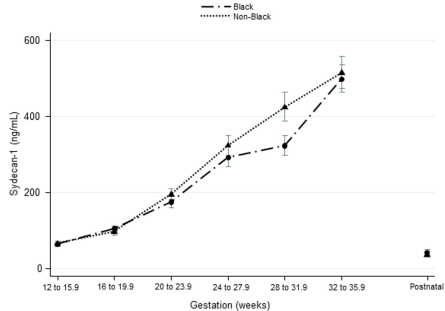
Number of samples						
Birthweight <3 <sup>rd</sup> centile	10	7	8	5	9	5
Birthweight 3 <sup>rd</sup> to 10 <sup>th</sup> centile	7	0	9	7	6	10
Birthweight >10 <sup>th</sup> centile	13	4	4	4	4	4

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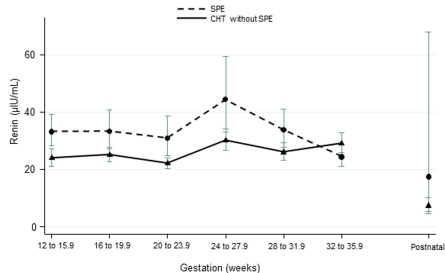
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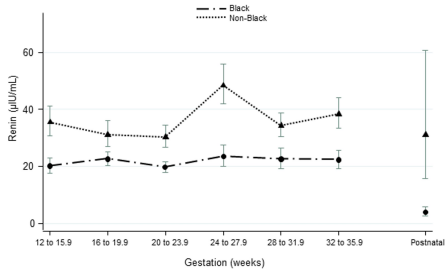
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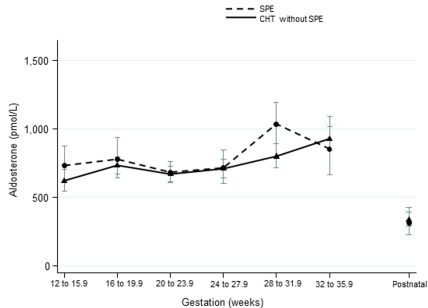
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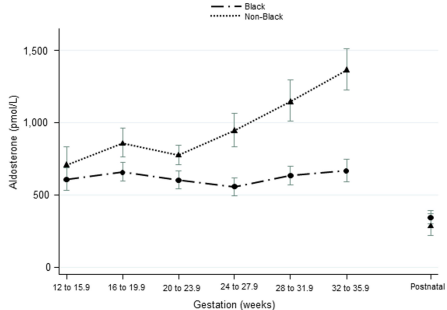
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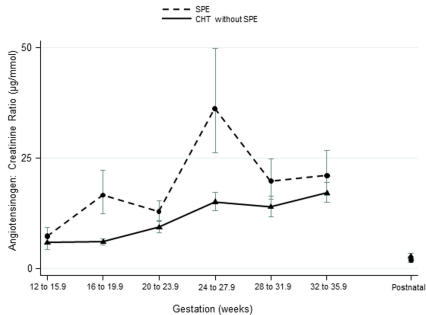
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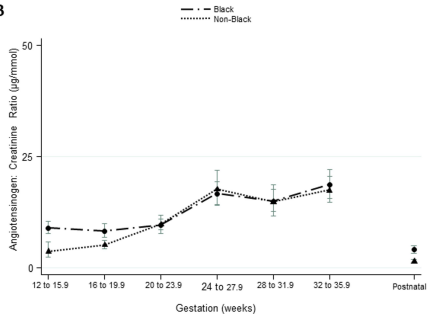
B



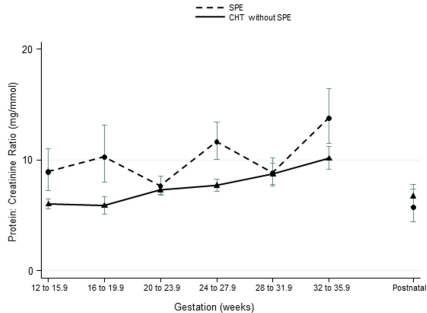
A



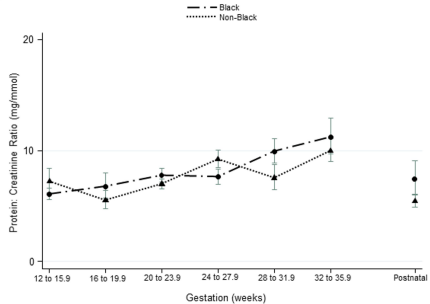
B



A



B

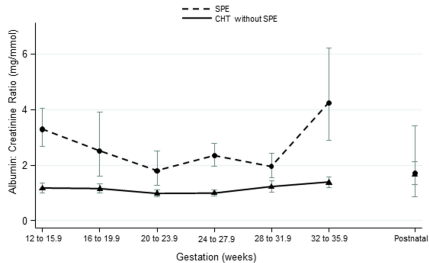


Number of samples							
SPE	6	4	8	8	8	7	3
CHT without SPE	21	25	14	15	14	14	11

Number of samples							
Black women	16	19	23	25	22	28	13
Non-Black women	11	10	29	19	20	23	8



A



B

